Supplemental methods

Chemicals

Unless specified all chemicals were purchased from Sigma (Sigma, St. Luis, MO). The solutions, such as ethanol, methanol and acetic acid were purchased from Fisher (Fisher Scientific, Pittsburg, PA).

Mice

Eight to 10 week old male mice, all on the C57Bl6 background were used. The C57Bl6 mice infused with PEG-catalase were purchased from Jackson laboratories (Jackson Laboratories, Bar Harbor, ME). For the rest of the experiments we used an established colony of catalase over-expressing mice and their wild type littermates generated and maintained in our laboratory. These mice over-express the human catalase gene specifically in the VSMCs, driven by the myosin heavy chain α promoter ($Tg^{cat\text{-}VSMC}$) and have been described previously[1].

Induction of AAA

AAA were induced with the application of $CaCl_2$ on the infrarenal aorta as described previously.[2] Briefly, 8-10 week old male mice on the C57Bl6 background were used. Mice were anesthetized with 1-2% isoflurane, the aorta was isolated from the inferior vena cava (IVC) using fine forceps, and a sterile gauze soaked in 0.25M sterile $CaCl_2$ was applied on its external surface for 15 minutes. A picture of the aorta was taken with a digital camera connected to the surgical microscope. The aortic diameter was measured in the midline between the renal arteries and the iliac bifurcation in live animals with a digital caliper and with image J (NIH) after taking a picture just prior to the application of the gauze and 8 weeks later before euthanizing each mouse. Buprenorphine at 0.05 mg/kg was used for post-operative analgesia. All the procedures were approved by the institutional animal care and use committee.

PEG-catalase infusion

In some experiments, mice were subjected to $CaCl_2$ surgery and infused intravenously with saline or PEG-catalase at a rate of 10,000 Units/Kg/day with an osmotic mini pump (Alzet, Cupertino, CA) connected to the jugular vein through a sterile catheter (Alzet, Cupertino, CA). The duration of PEG-catalase administration was 8 weeks, from 2 days prior to surgery until post-operative week 8. Because the maximum

duration of pump infusion is 4 weeks, the pumps were replaced with new ones after 28 days. In other experiments, wild type mice were first exposed to $CaCl_2$, and PEG-catalase or saline was infused from post-operative day 2 to post-operative day 12.

H_2O_2 quantification

H_2O_2 levels were measured using the Amplex Red assay (Life Technologies, Grand Island, NY). At each designated time point the infrarenal aortas were harvested and cleared from the surrounding fatty tissue on ice, cut into 2 mm rings and incubated for 50 min in Krebs Ringer's Phosphate Glucose (KRPG) buffer pH 7.4 at 37 ^{o}C. A H_2O_2 standard curve was generated with each assay. The levels of H_2O_2 were normalized per 2 mm aortic ring and expressed as pmoles/min/aortic ring.

Catalase activity

Catalase activity was measured in plasma and in aortas by quantifying the amount of H_2O_2 degradation over time using an Amplex Red based assay following the manufacturer's instructions (Life Technologies, Grand Island, NY). The aortas were washed extensively with 0.1 M Tris pH 7.4 before measuring catalase activity in order to remove completely the red blood cells, which contain high concentrations of catalase. Catalase activity in aortas was normalized per mg wet weight. For plasma catalase activity we used dilutions 1/25 in 0.1 M Tris pH 7.4. The blood was withdrawn from the right ventricle using a large bore needle to avoid hemolysis. The results obtained with this method were further confirmed using a different catalase activity assay (Cayman Chemical Company, Ann Arbor, MI), which utilizes the ability of catalase to oxidize methanol to formaldehyde.

Histology

Hematoxylin & Eosin, Verhoeff-Van Gieson, Masson's Trichrome, and Picrosirius Red staining were performed using standard protocols. Quantification of media thickness was done from hematoxylin and eosin stained sections on post-operative week 8. The media thickness was measured in each section at 3, 6, 9 and 12 o'clock under 100x magnification using image J (NIH, Bethesda, MD).

Zymography

For *in situ* gelatin zymography 7 μm frozen sections were prepared and stored at -80 ^{o}C until use. 1% LGT agarose (Sigma, St. Luis, MO) was prepared in Phosphate Buffered Saline (PBS) with and without 20 mmol/L EDTA. DQ Gelatin (Life Technologies, Grand Island, NY) was dissolved to 1 mg/ml in water and mixed 1:10 (vol/vol) with 1% LGT agarose. Forty (40) μl of the final solution was pipetted on each section, the slides were coverslipped and incubated for 10 min at 4 ^{o}C and then transferred into a 37^{o}C humid chamber for overnight incubation. Quantification of the fluorescent signal was performed using Image J (NIH, Bethesda, MD). For *in situ* elastin zymography, initially 1% LGT agarose was prepared in 50 mmol/L phosphate, 20 mmol/L EDTA buffer pH 5.5. DQ-elastin (Life Technologies, Grand Island, NY) was dissolved to 1mg/ml in water and diluted 1:10 with 1% LGT agarose/phosphate/EDTA solution in the presence or absence of 1 mmol/L of the cathepsin inhibitor N-Methoxysuccinyl-Ala-Ala-Pro-Val-chloromethyl ketone (Life Technologies, Grand Island, NY). Forty (40) μl of the final solution was applied on each section, agarose was gelled with a brief incubation at 4 ^{o}C and the sections were finally incubated at 37 ^{o}C for 48 hrs. Images were acquired with fluorescent microscopy.

Gelatin zymography was performed as described by Godin et al. [3] Briefly, mice were perfused with saline, the infrarenal aortas were dissected and placed in -80 ^{o}C until use. Each aorta was homogenized on ice with 150 μl lysis buffer (50 mM Tris, 150 mM NaCl, 10 mM $CaCl_2$, 0.1% SDS, 0.5% Brij 35, 1% Triton X-100, 0.05% azide pH 7.4), the lysates were spun at 10000xg for 15 min at 4 ^{o}C and the supernatants were collected. Protein concentration was specified using the Bradford method. Twenty (20) μg of lysates were loaded on each lane of a premade 10% zymogram gel (Bio-Rad, Hercules, CA), then SDS was exchanged with Triton X-100 for 1 hr at room temperature and the gel was incubated with zymogram development buffer (Bio-Rad, Hercules, CA) for 2 days at 37 ^{o}C with slight rocking. Next, the gel was stained with Colloidal Brilliant Blue (Bio-Rad, Hercules, CA) for 45 min and destained with destaining buffer (60% water, 30% methanol, 10% acetic acid) for 1 hr.

Immunohistochemistry and Westen Blotting

Paraffin embedded aortic sections were stained using antibodies for cleaved caspase 3 (9661S, dilution 1:50, Cell Signaling Technology, Inc. Danvers, MA), the macrophage marker mac3 (cat#550292, dilution 1:100, Pharmingen, San Diego, CA) the lymphocyte marker CD3 (Ab5690, dilution 1:100, Abcam, Cambridge, MA), the lipid peroxidation produce HNE (cat# 393207, dilution 1:100, Millipore, Billerica, MA), and smooth muscle α-actin (A5691 dilution 1:50, Sigma, St. Luis, MO). Frozen sections were used to stain for neutrophils (Ab2557, dilution 1:100, Abcam, Cambridge, MA)

and smooth muscle α-actin (A5691, dilution 1:50, Sigma, St. Luis, MO) using standard protocols.

Western blotting for superoxide dismutase 1 and glutathione peroxidase 1&2 was performed using rabbit polyclonal antibodies against superoxide dismutase 1 (sc-11407, dilution 1:200, Santa Cruz Biotechnology, Inc. Dallas, TX), and glutathione peroxidase ½ (sc30147, dilution 1:100, Santa Cruz Biotechnology, Inc. Dallas, TX) using standard protocols. The antibodies were incubated overnight. The secondary antibody was a polyclonal anti-rabbit antibody conjugated with HRP (Bio-Rad, Hercules, CA) and used in dilution 1/2500. Films were developed using ECL.

Apoptosis & Proliferation

We used Terminal deoxynucleotidyl transferase mediated dUTP Nick End Labeling (TUNEL) staining on paraffin embedded sections to assess for apoptotic cell death following the manufacturer's protocol (Roche Applied Science, Indianapolis, IN). Images were acquired with fluorescent microscopy. To quantify apoptotic cells we counted the number of TUNEL positive cells in the intima and media per aortic section under 100x magnification using ImageJ (NIH, Bethesda, MD). In addition, we quantified the TUNEL positive area over the total vessel area using image J (NIH, Bethesda, MD). To assess for proliferation, mice were injected with 100 mg/Kg solution of 5-bromo-2'-deoxyuridine (BRDU) in saline for 2 consecutive days before they were euthanized. Staining for proliferating cells were performed in paraffin sections using an antibody against BRDU (Ab1893, Abcam, Cambridge, MA).Quantification of proliferation was performed by counting the number of BRDU positive cells per aortic section, as well as by calculating the percent BRDU positive area using image J (NIH, Bethesda, MD).

Real time PCR

Animals were euthanized with CO_2 at the designated time point and perfused with cold saline. The infrarenal aortas were harvested, quickly cleaned from the surrounding fat tissues and snapped frozen in liquid nitrogen until use. The mRNA was isolated using an RNA isolation kit (Qiagen, Valencia, CA) and RT reactions were carried out from 0.5 μg of starting mRNA material using Superscript III (Life Technologies, Grand Island, NY). Following purification of cDNA with a commercial kit (Qiagen, Valencia, CA), real time PCR reactions were performed 18s,TNFα, OPN, MCP-1, CD68, IL-1β, TGFβ1, TGFβ2, MMP-2, MMP-9, IL-6, ICAM-1, SDF-1, catalase, Superoxide Dismutase 1, glutathione peroxidase 1, peroxiredoxin1, and peroxiredoxin2 using mouse specific primer mix (Qiagen, Valencia, CA). Human catalase primer mix was also purchased

from Qiagen. Copy number for each marker was normalized per 10^6 copies of the housekeeping gene 18s.

Statistical analysis

Data are presented as mean±SEM. Due to the relatively small number of animals the nonparametric Mann-Whitney test was used to examine statistical significance in this study. For multiple groups comparison one way ANOVA was employed. To compare multiple groups at multiple time points two way ANOVA was performed. Statistical tests were performed using GraphPad Prism v.5.00 (GraphPad Software, San Diego CA) and SPSS v.20 (IBM Corporation, Armonk, NY).

Supplemental references

1. Zhang Y, Griendling KK, Dikalova A, Owens GK, Taylor WR. Vascular hypertrophy in angiotensin II-induced hypertension is mediated by vascular smooth muscle cell-derived H2O2. *Hypertension.* 2005;46:732-737.

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